

The invention claimed is:

1. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-polar
chromatographic surfaces and eluting said mixture of polynucleotides with a
mobile phase comprising a counterion agent and an organic solvent,
wherein said monolith is contained within a fused silica tube having an inner
diameter in the range of 1 micrometer to 1000 micrometer,
wherein said monolith is immobilized by covalent attachment at the inner wall of
said tube, and
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene)
matrix.

2. A method of claim 1 wherein said tube is devoid of retaining frits.

3. A method of claim 1 wherein said monolith is characterized by having 100,000 to
200,000 theoretical plates per meter.

4. A method of claim 3 wherein said theoretical plates per meter is determined from the
retention time of single stranded p(dT)₁₈ standard using the following equation:

$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)^2$$

wherein N is the number of theoretical plates, t_R is the retention time of said
standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half
height, and L is the length of the monolith in meters.

5. A method of claim 4 wherein said tube has an inner diameter of 200 micrometer and
a length of 60 mm, wherein during said isocratic elution said monolith has a back
pressure in the range of 180 to 200 bar, and a flow rate in the range of 2 to 3 $\mu\text{L}/\text{min}$
at an elution temperature of 50°C.

6. A method of claim 1 wherein said mobile phase is devoid of EDTA.

7. A method of claim 1 wherein said monolith has a surface morphology, as determined
by scanning electron microscopy, that resembles the surface morphology of
octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface
morphology of said monolith is brush-like.

8. A method of claim 1 wherein said monolith has a surface morphology, as determined
by scanning electron microscopy, that resembles the surface morphology of

901.5, 4.0 amu isolation width, 19% relative collision energy; scan, 250-1810 amu; electrospray voltage, 3.2 kV; sheath gas, 30 units; sheath liquid, acetonitrile; flow rate, 3.0 μ L/min; sample, 25 pmol raw product.

- 5 While the foregoing has presented specific embodiments of the present invention, it is to be understood that these embodiments have been presented by way of example only. It is expected that others will perceive and practice variations which, though differing from the foregoing, do not depart from the spirit and scope of the invention as described and claimed herein.

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octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

9. A method for separating a mixture of polynucleotides, said method comprising:

applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube, and wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

10. A method of claim 9 wherein said monolith is contained within said fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer.

11. A method of claim 9 wherein said tube is devoid of retaining frits.

12. A method of claim 9 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

13. A method of claim 9 wherein said mobile phase is devoid of EDTA.

14. A method of claim 9 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

15. A method of claim 9 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

16. A method for separating a mixture of polynucleotides, said method comprising:

applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube,

wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer,

wherein said tube is devoid of retaining frits, and

wherein said polynucleotides comprise double-stranded fragments having lengths in the range of 3 to 600 base pairs.

17. A method of claim 16 wherein said mobile phase is devoid of EDTA.

18. A method of claim 17 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

19. A method of claim 16 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

20. A method of claim 16 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

21. A method of claim 16 wherein said monolith is characterized by having at least 100,000 theoretical plates per meter.

22. A method of claim 16 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

23. A method for separating a mixture of polynucleotides, said method comprising: applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix, wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter, wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, and wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

24. A method of claim 23 wherein said theoretical plates per meter is determined from the retention time of single stranded p(dT)₁₈ standard using the following equation:

$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)^2$$

wherein N is the number of theoretical plates, t_R is the retention time of said standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half height, and L is the length of the monolith in meters.

- 5 25. A method of claim 23 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like
26. A method of claim 23 wherein said tube is silianized.
- 10 27. A method of claim 23 wherein said tube is devoid of retaining frits.
28. A method of claim 23 wherein said mobile phase is devoid of EDTA.
29. A method of claim 23 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.
- 15 30. A method for separating a mixture of polynucleotides, said method comprising: applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said mobile phase is devoid of EDTA, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix
- 20 31. A method of claim 30 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.
32. A method of claim 30 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.
- 30 33. A method of claim 32 wherein said tube is devoid of retaining frits.

34. A method of claim 30 wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter.
35. A method of claim 30 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.
36. A method of claim 30 wherein said tube has been silanized.
37. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent, wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.
38. A method of claim 37 wherein said mobile phase is devoid of EDTA.
39. A method of claim 37 wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer.
40. A method of claim 37 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.
41. A method of claim 37 wherein said tube is devoid of retaining frits.
42. A method of claim 37 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.
43. A method of claim 37 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.
44. A method for separating a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-polar chromatographic surfaces and eluting said mixture of polynucleotides with a mobile phase comprising a counterion agent and an organic solvent,

wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,

wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer,

5 wherein said monolith is immobilized at the inner wall of said tube,

wherein said tube is devoid of retaining frits.

45. A method of claim 44 wherein said mobile phase is devoid of EDTA.

46. A method of claim 44 wherein said monolith is contained within a tube having an inner diameter in the range of 10 micrometer to 300 micrometer.

10 47. A method of claim 44 wherein said monolith is immobilized at the inner wall of said tube and wherein said tube has been silanized.

48. A method of claim 44 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein
15 said surface morphology of said monolith is brush-like.

49. A method of claim 44 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

20 50. A method of claim 44 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles; wherein said surface morphology of said monolith is rugulose.

25 51. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer, wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

30 52. A device of claim 51 wherein said tube is devoid of retaining frits.

53. A device of claim 51 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

35 54. A device of claim 53 wherein said theoretical plates per meter is determined from the retention time of single stranded p(dT)₁₈ standard using the following equation:

$$(N/L) = (5.54/L) \left(\frac{t_R}{w_{0.5}} \right)^2$$

wherein N is the number of theoretical plates, t_R is the retention time of said standard determined during an isocratic elution, $w_{0.5}$ is the peak width at half height, and L is the length of the monolith in meters.

- 5 55. A device of claim 54 wherein said tube has an inner diameter of 200 micrometer and a length of 60 mm, wherein during said isocratic elution said monolith has a back pressure in the range of 180 to 200 bar, and a flow rate in the range of 2 to 3 $\mu\text{L}/\text{min}$ at an elution temperature of 50°C .
- 10 56. A device of claim 51 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.
- 15 57. A device of claim 51 wherein the chromatographic surfaces of said monolith are devoid of micropores.
58. A device of claim 57 wherein said monolith has channels sufficiently large for convective flow of said mobile phase.
- 20 59. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
wherein said monolith is contained within a fused silica tube, and
wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.
- 25 60. A device of claim 59 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.
61. A device of claim 59 wherein said tube is devoid of retaining frits.
62. A device of claim 59 wherein said monolith is characterized by having 10,000 to 200,000 theoretical plates per meter.
- 30 63. A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface

morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

64. A device of claim 59 wherein said monolith comprises an underivatized monolithic stationary phase.

5 65. A device of claim 59 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

10 66. A device of claim 59 wherein said monolith is devoid of micropores and wherein said monolith has channels sufficiently large for convective flow of said mobile phase.

15 67. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene) matrix,
wherein said monolith is contained within a fused silica tube,
wherein said tube has been silanized, and
wherein said tube is devoid of retaining frits.

20 68. A device of claim 67 wherein said monolith is immobilized by covalent attachment at the inner wall of said tube.

69. A device of claim 67 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

25 70. A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

71. A device of claim 67 wherein said tube has an inner diameter in the range of 1 micrometer to 1000 micrometer.

30 72. A device of claim 67 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

35 73. A device for separating a mixture of polynucleotides, said device comprising:

a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene)
matrix,

wherein said monolith is contained within a tube having an inner diameter in the
range of 1 micrometer to 1000 micrometer,

wherein said monolith is characterized by having 10,000 to 200,000 theoretical
plates per meter.

74. A device of claim 73 wherein said monolith is contained within a tube having an
inner diameter in the range of 1 micrometer to 1000 micrometer.

75. A device of claim 73 wherein said monolith is immobilized by covalent attachment
at the inner wall of said tube.

76. A device of claim 75 wherein said tube is devoid of retaining frits.

77. A method of claim 73 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein
said surface morphology of said monolith is brush-like.

78. A method of claim 73 wherein said monolith has a surface morphology, as
determined by scanning electron microscopy, that resembles the surface
morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein
said surface morphology of said monolith is rugulose.

79. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene)
matrix,

wherein said monolith is characterized by having at least 100,000 theoretical
plates per meter,

wherein said monolith is contained within a silanized fused silica tube having an
inner diameter in the range of 10 micrometer to 1000 micrometer,

wherein said monolith is immobilized at the inner wall of said tube.

80. A device of claim 79 wherein said monolith is characterized by having 100,000 to
200,000 theoretical plates per meter.

81. A device of claim 79 wherein said monolith is contained within a tube having an
inner diameter in the range of 1 micrometer to 1000 micrometer.

82. A device of claim 79 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is brush-like.

5 83. A device of claim 82 wherein said tube is devoid of retaining frits.

84. A device of claim 79 wherein said monolith has a surface morphology, as determined by scanning electron microscopy, that resembles the surface morphology of octadecyl modified poly(styrene-divinylbenzene) particles, wherein said surface morphology of said monolith is rugulose.

10 85. A miniaturized chromatographic system for separating a mixture of polynucleotides, said system comprising the device of claim 79.

86. A device for separating a mixture of polynucleotides, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith has a surface morphology, as determined by scanning
electron microscopy, that resembles the surface morphology of octadecyl
modified poly(styrene-divinylbenzene) particles, wherein said surface
morphology of said monolith is rugulose and brush-like,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene)
matrix,

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20 wherein said monolith is contained within a fused silica tube having an inner diameter in the range of 1 micrometer to 1000 micrometer,
wherein said monolith is immobilized at the inner wall of said tube.

87. A device of claim 86 wherein said tube is devoid of retaining frits.

25 88. A device of claim 86 wherein said monolith is characterized by having 100,000 to 200,000 theoretical plates per meter.

89. A device of claim 86 wherein said tube has been silanized.

90. A device of claim 86 wherein said surfaces of said monolith are non-porous.

30 91. A device of claim 86 wherein said monolith is formed from a polymerization mixture including underivatized styrene, a crosslinking agent, and a porogen, wherein said porogen comprises tetrahydrofuran.

92. A device of claim 86 wherein said polynucleotides comprise double-stranded fragments having lengths in the range of 3 to 600 base pairs.

93. A method of claim 16 including analyzing eluted polynucleotides by mass spectral analysis.

94. A method of claim 23 including analyzing eluted polynucleotides by mass spectral analysis.

95. A system of claim 85 wherein said monolith is operatively coupled to a mass spectrometer.

5 96. A method for desalting a mixture of polynucleotides, said method comprising:
applying said mixture of polynucleotides to a polymeric monolith having non-polar
chromatographic surfaces and eluting said mixture of polynucleotides with a
mobile phase comprising a counterion agent and an organic solvent,
10 wherein said monolith is characterized by having 100,000 to 200,000 theoretical
plates per meter,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene)
matrix,
wherein said monolith is contained within a fused silica tube having an inner
diameter in the range of 1 micrometer to 1000 micrometer,
15 wherein said monolith is immobilized at the inner wall of said tube.

97. A chromatographic device, said device comprising:
a polymeric monolith having non-polar chromatographic surfaces,
wherein said monolith comprises an underivatized poly(styrene-divinylbenzene)
matrix,
20 wherein said monolith is contained within a silanized fused silica tube having an
inner diameter in the range of 10 micrometer to 1000 micrometer, and
wherein said monolith is immobilized at the inner wall of said tube.